Accurate timetrees do indeed require accurate calibrations. Response to comment by Hedges et al.

Jennifer L. Morris[1], Mark N. Puttick[1,2], James Clark[1], Dianne Edwards[4], Paul Kenrick[2], Silvia Pressel[3], Charles H. Wellman[5], Ziheng Yang[6], Harald Schneider[1,3,7,*] Philip C. J. Donoghue[1,*]

[1]School of Earth Sciences, University of Bristol, Life Science Building, Tyndall Avenue, Bristol, BS8 1TQ.

[2]Departments of Earth and [3]Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD.

[4]School of Earth and Ocean Sciences, Cardiff University, Main Building, Park Place, Cardiff, CF10

[5]Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield, S10 2TN.

[6]Department of Genetics, Evolution and Environment, University College London, Gower Street, London WC1E 6BT, UK

[7]Xishuangbanna Tropical Botanical Garden, Centre for Integrative Conservation, Chinese Academy of Sciences, China

*authors for correspondence

We (1) attempted to establish an evolutionary timescale for land plant evolution utilizing available genome scale data and a new set of calibrations constraining the age of clades based on critical analysis of palaeontologic, phylogenetic (2), and geologic evidence. We explored many factors, such as the inclusion or exclusion of a calibration on the crown-embryophyte node and concluded that the living clade of land plants emerged in a middle Cambrian – Early Ordovician interval.

Hedges and colleagues (3) argue that the results of our study are not robust to dating strategies since removal of maximum constraints (maxima) results in significantly older clade age estimates. They conducted experiments by removing Paleozoic maxima and all clade age constraints bar for spermatophytes. Their justifications for such experiments are that (i) examples abound of taxa missing as fossils for most of their history, and (ii) clade history may be geographically restricted or not accessible in today's sedimentary record. The crux of their argument is the veracity of maxima on clade ages. Hedges and colleagues imply that maxima are applied either arbitrarily (4) or through a literal reading of the fossil record. This is not the approach we employed; our maxima were based on fossil occurrence and absence, as well as the structure of the stratigraphic record (5)

As one example, the maximum constraint on the age of crown-embryophytes is reliable according to Hedges et al.'s definition. Terrestrial Silurian land plant spores are also known to occur alongside marine algal cysts which are similarly composed of sporopollenin, an inert, effectively indestructible biological polymer. Thus, marine algal cysts, which are sampled worldwide deep into the Proterozoic, serve as a taphonomic control on land plant spores in marine sequences: presence of algal cysts in the absence of land plant spores indicates an environment compatible with the preservation of land plant spores, hence, our 515.5 Ma maximum for crown-embryophytes.

The results Hedges et al. present are unsurprising: as times and rates are confounded in clock dating analysis, fossil calibrations (and in particular maximum age constraints) are of utmost importance, and if we remove the maxima, the age estimates are likely to increase (6). However, Hedges and colleagues do not consider the evidence we presented for the choice of maxima, simply presuming that the constraints are inherently unreliable. Further, their results differ from ours principally in their decreased precision. Even after removing four constraints, all but one of their clade age estimates overlap with ours; when they remove all maxima, their clade age estimates overlap in 12/20 highlighted cases.

Morris et al. (1) present a timescale for the evolutionary emergence of land plants that goes significantly beyond common practice in exploring parameter space and integrating uncertainty, built on calibrations that follow best practice (7). We see no evidence that would result in a deep Proterozoic origin of land plants envisaged by analyses based on out-moded strict clock methods (e.g. 8, 9).

1. Morris JL*, et al.* (2018) The timescale of early land plant evolution. *Proceedings of the National Academy of Sciences*.
2. Puttick MN*, et al.* (2018) The interrelationships of land plants and the nature of the ancestral embryophyte. *Current Biology* 28:1-13.
3. Hedges SB, Tao Q, Walker M, & Kumar S (2018) Accurate timetrees require accurate calibrations. *Proceedings National Academy of Sciences*.
4. Ho SYW & Phillips MJ (2009) Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Systematic Biology* 58:367-380.
5. Warnock RC, Parham JF, Joyce WG, Lyson TR, & Donoghue PC (2015) Calibration uncertainty in molecular dating analyses: there is no substitute for the prior evaluation of time priors. *Proceedings of the Royal Society B: Biological Sciences* 282(1798):20141013.
6. dos Reis M, Donoghue PCJ, & Yang Z (2016) Bayesian molecular clock dating of species divergences in the genomics era. *Nature Reviews Genetics* 17:71-80.
7. Parham JF*, et al.* (2012) Best practices for justifying fossil calibrations. *Syst Biol* 61(2):346-359.
8. Heckman DS*, et al.* (2001) Molecular evidence for the early colonization of land by fungi and plants. *Science* 293(5532):1129-1133.
9. Hedges SB, Blair JE, Venturi ML, & Shoe JL (2004) A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evolutionary Biology* 4(2).